STUDIES ON AN AMINO ACID DEGRADATION PRODUCT OF STREPTOTHRICIN

BY

CHARLES ALLEN WEST

A.B., DePauw University, 1949

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1952

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INTRODUCTION

Streptothricin, an antibiotic substance isolated from the culture filtrates of *Streptomyces lavendulae,* yields three ninhydrin-positive compounds when hydrolyzed with hydrochloric acid. These three materials are detected at R_f values 0.30, 0.16 and 0.06 on filter paper chromatograms developed with phenol-water and sprayed with ninhydrin reagent. The purpose of this work was the separation of these three components, and the purification and chemical characterization of Compound B, the substance of R_f value 0.16.

DISCUSSION OF RESULTS

The acid hydrolysis mixture of streptothricin was separated on a 'Solka Floc' chromatogram developed as a flowing chromatogram with *t*-butanol-acetic acid-water (2-1-1). Compound B was isolated from appropriate fractions as the flavianate salt which was recrystallized from hot water. The flavianate salt was converted to the hydrochloride by exchange on a column of Amberlite IR-45 anion exchange resin (chloride phase). The hydrochloride of B was purified by recrystallization from a dilute hydrochloric acid-methanol solution by the addition of acetone. It melts over a range of 173-190° with decomposition. It analyzes most correctly for a structure of $C_6H_{12}N_4O_3 \cdot 2HCl$, and possesses a specific rotation of +78.9° (22°, D, c 2.35, in water).

Compound B dihydrochloride shows absorption maxima in the infrared at 1725 cm.$^{-1}$, 1682 cm.$^{-1}$ and 1505 cm.$^{-1}$ characteristic of a carboxylic acid, a carbon to nitrogen double-bond, and an amino acid, respectively. It shows only end absorption in the ultraviolet. Data from potentiometric titrations with standard alkali indicated a minimal molecular weight for B of 266-274, and two groups of pK_a 2.30 and pK_a 8.80. Titration in 66 per cent dimethylformamide solution indicated an additional basic group of pK_a 12.65.

A Van Slyke nitrous acid determination demonstrated that one of the four nitrogens in B was primary amino nitrogen, and a ninhydrin-carbon dioxide determination revealed that this primary amino group was not alpha to a carboxyl group. The Pauly test for imidazoles gave an atypical positive response with B, whereas, the Sakaguchi test for certain types of guanidine groups and the bi-

acetyl-monoxime test for ureido compounds were negative. The cobaltic hydroxide complexing test gave a color most closely resembling that obtained with lactic acid.

Benzoylation of Compound B proceeded in an atypical manner to produce both an alkali-soluble and an alkali-insoluble derivative.

Hydrolysis of Compound B with 0.33*N* sodium hydroxide liberated two moles of ammonia after 140 hours. A partial hydrolysis of B with barium hydroxide (34 hours, 79-84°) gave rise to at least three different ninhydrin-positive products. The reaction solution also gave a strong positive response with the biacetyl-monoxime test for ureido compounds. The products were separated on a 'Solka Floc' column using 3-1 phenol-water as the developing solvent system, but were not identified.

One mole of Compound B reduced four moles of periodate. The first mole of periodate was reduced rapidly at pH 4.6 and at pH 8.5, whereas, the second and third moles were reduced more rapidly at the alkaline pH. The fourth mole was reduced slowly at both pH values. One mole of ammonia, one mole of formaldehyde and two-three moles of formic acid were produced during the total oxidation of B with periodate.

The results seem most consistent with a structure for B containing a carboxyl group, a primary amino group, a cyclic guanido group and a hydroxyl group substituted on a four carbon chain.

SUMMARY

1. The three ninhydrin-positive compounds arising during an acid hydrolysis of streptothricin were separated on a 'Solka Floc' (powdered cellulose) chromatogram using *t*-butanol-acetic acid-water as the developing solvent system.

2. Compound B, one of these degradation products, was purified by the preparation of its crystalline flavianate and hydrochloride salts.

3. Physical and chemical properties were determined for Compound B.

4. A proposed type of structure for Compound B based on this physical and chemical data includes a cyclic guanido group, a primary amino group, a hydroxyl group and a carboxyl group substituted on a four carbon chain.

VITA

The author was born in Greencastle, Indiana, on November 4, 1927. He attended elementary schools in that community and graduated from Greencastle High School in June, 1945. From July, 1945, until June, 1949, he attended DePauw University in Greencastle with the assistance of a Rector Scholarship. After receiving the degree of Bachelor of Arts from that institution, he enrolled in the Graduate College of the University of Illinois in September, 1949. From the time of entrance until June, 1950, he held a Teaching Assistantship, and from September, 1950, until August, 1952, he held a Research Fellowship jointly sponsored by the Upjohn Co., Eli Lilly and Co., and the Abbott Laboratories.